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Environmental Toxicology and Chemistry, Vol. 16, No. 4, pp. 742–753, 1997
Printed in the USA
0730-7268/97 $6.00 + .00

ACCUMULATION AND TOXICOKINETICS OF FLUORANTHENE IN SEDIMENT BIOASSAYS WITH FRESHWATER AMPHIPODS

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(*Received* 16 *January* 1996; *Accepted* 22 *August* 1996)

Abstract—Two freshwater amphipods, *Hyalella azteca* and *Diporeia* sp., were exposed to sediment spiked with radiolabeled fluoranthene at nominal concentrations of 0.1 (trace) to 1,270 nmol fluoranthene/g dry weight. In two experiments, uptake kinetics and mortality were determined over 30-d exposures. Concentrations of fluoranthene in sediment and pore water were also measured. Mean survival of *H. azteca* was generally high, greater than 90% after 10 or 16 d, and greater than 74% after 30 d. Mean survival was lower for *Diporeia*, 14% after a 30 d exposure to the highest sediment concentration in experiment 1, and 53% in experiment 2. Tissue concentrations in *Diporeia* were as high as 2 to 4 μmol/g wet weight, a body burden that could be expected to result in death by narcosis. *Hyalella azteca* did not typically accumulate more than 1 μmol/g wet weight, which is consistent with the lower observed mortality. Apparent steady-state biota–sediment accumulation factors (BSAFs, lipid- and organic carbon-normalized) for sediment concentrations other than trace level tended to be higher for *Diporeia* (0.345–0.818) than for *H. azteca* (0.161–0.612). The BSAFs for trace levels tended to be lower for both species (0.045–0.436) in comparison to higher sediment concentrations. For both organisms, the internal concentration based on body residue was a more reliable indicator of toxicity than were equilibrium partitioning predictions.

Keywords—Fluoranthene Sediment Amphipods Toxicokinetics Accumulation

INTRODUCTION

The equilibrium partitioning (EqP) approach has been proposed as a method for the development of sediment quality criteria [1]. This approach predicts biological effects of hydrophobic compounds on the basis of their organic-carbon-normalized concentration in the sediment. Equilibrium partitioning predicts that biological effects based on pore-water concentrations can be estimated from effects determined in water-only exposures, because the chemical activity of a compound in sediment will be reflected by its freely dissolved interstitial water concentration. Toxicity bioassays with benthic invertebrates, which have been used to test predictions of sediment toxicity, have, in general, confirmed the utility of the approach [1–3].

Another approach for evaluating contaminant exposure is the use of the critical body residue (CBR) method [4]. This method predicts that, for chemicals that act by narcosis (most nonpolar organics), the potency measured at the site of toxic action should be essentially constant for similar organisms [5,6]. The rationale for the CBR method is based on the lipid theory of narcosis and a later refinement, the volume fraction theory of narcosis (reviewed in [7]). These theories propose that narcosis results from physical modification or deformation of the phospholipid membrane by adsorption of hydrophobic compounds. Narcosis is thought to occur when a sufficient amount of compound, on the basis of molar concentration or volume fraction, has been adsorbed to produce a disruption of membrane function. Thus, the narcotic effect of a range of organic chemicals should be observed at a fairly constant concentration in hydrophobic tissues. The CBR approach, which has been validated with a variety of organisms, including aquatic vertebrates [8–10] and invertebrates [11–13], predicts that acute narcosis for hydrophobic organic chemicals will occur at body burdens of 2 to 8 μmol/g wet weight tissue [9]. The present work attempts to determine whether there is a reliable relationship between body burden of the hydrophobic, polycyclic aromatic hydrocarbon, fluoranthene, and a toxic effect, mortality in this case, for two species of freshwater amphipods.

Some toxicokinetic studies have raised questions about the potential for kinetic limitations to bioaccumulation of sediment-associated polycyclic aromatic hydrocarbons (PAHs). Specifically, when the freshwater benthic amphipod, *Diporeia* sp., was exposed to a mixture of PAHs or a single compound (pyrene) sorbed to sediments, the observed toxicity was approximately 10 times lower than would be predicted by equilibrium partitioning [12,13]. The low toxicity observed in this species was apparently not the result of reduced sensitivity in *Diporeia*, because the body burden required to produce a toxic effect in these experiments (6–9 μmol/g tissue) was in the expected range (2–8 μmol/g tissue) for a nonpolar narcotic compound [4]. In other experiments, the apparent biota–sediment accumulation factors (BSAFs, lipid-normalized tissue concentration divided by the organic-carbon-normalized sediment concentration) were observed to be substantially lower for PAHs than for chlorinated hydrocarbons [14]. The low BSAF values for the PAHs were not due to biotransformation because *Diporeia* has been shown to have, at most, a very limited ability to biotransform PAHs [15].

This apparent limitation in the bioavailability of PAHs was not observed in the toxicity tests used for verifying the EqP approach and setting sediment criteria [1]. Several factors may have contributed to the differences between the results of the

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verification efforts and the observations with *Diporeia*. First, differences in sediment chemistry, even after organic carbon normalization, may contribute to residual variability in the bioavailability between sediments [16]. Second, verification was performed with the freshwater amphipod, *Hyalella azteca*, rather than with *Diporeia*, and there may be behavioral or other differences between the two amphipods that modify the exposure. For example, organisms that avoid the sediment can experience lower exposure [17]. Finally, factors such as temperature may influence the rate of accumulation and time to steady state. For example, *Diporeia* exhibits a more rapid accumulation rate with pyrene and chrysene and a shorter time to steady state at 10°C, compared to 4°C [13,18]. Amphipod species that are typically exposed at higher temperatures, such as *H. azteca* (20–25°C) and *Rhepoxynius abronius* (15°C), may accumulate a toxic dose more rapidly than *Diporeia* (4°C). Because the methods used for validating the EqP approach did not study the kinetics of accumulation, the reasons for the differences between *Diporeia* and other test species remain unknown.

The present study compares the relative sensitivity of the standard freshwater amphipod test species, *H. azteca*, to *Diporeia* sp., based on actual body burdens and observed toxic effects of sediment-associated fluoranthene. *Diporeia* spp. are found in deep lakes of North America [19] and are the dominant macrobenthic invertebrates of the Great Lakes, where they tend to feed on bacteria-rich sediments. Previously known as *Pontoporeia hoyi*, a recent taxonomic reassessment transferred this amphipod to a new genus, *Diporeia* [19]. The exact number of species is uncertain and remains under investigation. Life span ranges from 1 to 3 years [20]. *Hyalella azteca*, an epibenthic detritovore that burrows into surface sediment, is found in lakes, ponds, and streams throughout North and South America (see references in [21]).

A better understanding of the relative kinetics of bioaccumulation in conjunction with observations of effects and sediment bioavailability should help to resolve questions concerning differences between studies with Great Lakes sediments and organisms and those that have been performed with other sediments and organisms. A second goal of these experiments was to establish critical body burdens for the narcotic effect of fluoranthene in these species.

MATERIALS AND METHODS

Chemicals

Radiolabeled [3-^{14}C]fluoranthene was purchased from Chemsyn Laboratories (Lenexa, KS, USA) for the first experiment (55 mCi/mmol) and from Sigma Chemical Co. (St. Louis, MO, USA) for the second experiment (45 mCi/mmol). Unlabeled fluoranthene was purchased from Aldrich Chemical Co. (Milwaukee, WI, USA). The [^{14}C]fluoranthene was determined to be 98% pure by thin-layer chromatography (benzene/hexane, 20:80, v/v) on silica plates (Alltech Associates, Deerfield, IL, USA). Silica gel for column chromatography was obtained from Fisher Scientific Co. (Pittsburgh, PA, USA).

Separate stock solutions were prepared for each sediment concentration by combining [^{14}C]fluoranthene with unlabeled fluoranthene in acetone. The concentration of total fluoranthene in each stock solution was determined by gas chromatography/mass spectrometry-selected ion monitoring (GC/MS-SIM) using a Hewlett Packard model 5980 series II gas chromatograph equipped with a model 5971 mass selective detector (Hewlett Packard, San Fernando, CA, USA)[22]. The concentration of radiolabeled fluoranthene in each stock solution was determined by liquid scintillation counting (LSC) on a Tri-Carb Liquid Scintillation Analyzer (Model 2500 TR, Packard Instrument Co., Meridien, CT, USA). Samples were corrected for quench using the external standards ratio method after subtracting background. Triplicate samples from each stock solution were analyzed by LSC and the mean value used to calculate the specific activity of each stock solution (μCi of radiolabeled fluoranthene/μmol of total fluoranthene as determined from GC/MS). Concentrations of total fluoranthene (radiolabeled and unlabeled) in the sediment and tissue samples were calculated from the amount of radioactivity in the samples and the specific activity of each stock solution (as determined by GC/MS and LSC, described above). Sample concentrations are presented as total fluoranthene equivalents on a molar basis.

Experimental design

Two experiments were conducted that exposed *H. azteca* and *Diporeia* to [^{14}C]fluoranthene-dosed sediment for up to 30 d. To avoid the potential for photoinduced toxicity, all experiments were conducted under yellow light (>500 nm wavelength) and the mode of action was expected to be nonpolar narcosis. Sediment concentrations were selected on the basis of the toxicokinetics of pyrene ([13], and see Discussion), which has a log K_{ow} of 5.2, which is similar to that of fluoranthene (log K_{ow} = 5.09) [23], and median lethal concentration (LC50) values for *H. azteca* from the literature ([24,25], and see Discussion). Nominal sediment concentrations for the first experiment were 0 (control), 0.1 (trace), 40, 80, 160, and 320 nmol fluoranthene/g dry weight sediment for *H. azteca*, and 0 (control), 0.1 (trace), 160, 320, 630, and 1,270 nmol/g dry weight for *Diporeia* in both experiments (mol. wt. = 202.3 g/mol). *Hyalella azteca* were exposed to the same fluoranthene concentrations as *Diporeia* in the second experiment. Uptake kinetics and mortality were determined by sampling animals after 1, 2, 4, 10, 17, and 30 d of exposure in the first experiment and 1, 2, 5, 10, 16, and 30 d in the second experiment. For each species, five beakers from each concentration (including controls) were analyzed on days 10 and 30. Triplicate beakers were analyzed for each species and each concentration (not including controls) at other time points. The trace exposures for each species served as controls for mortality at the intermediate time points.

Sediment spiking

Sediment was collected by ponar grab from a 45-m-deep station (43.03°N, 86.37°W) in Lake Michigan. Low concentrations of PAHs were found in sediments of this station [26]. The sediment was sieved (1 mm Nytex, Tetco, Briarcliff Manor, NY, USA) and stored at 4°C until dosed. For the first experiment, sediment was shipped to the U.S. Environmental Protection Agency (EPA) Newport, Oregon, and spiked according to the EPA-recommended rolling jar method described below [27]. For the second experiment, sediment was spiked at the Great Lakes Environmental Research Laboratory (GLERL) using the same protocol. Stock solutions were evaporated onto the inside of 3.8-L (1-gallon) glass jars. Sediment (2,400 to 2,600 g wet weight) and filtered (0.45 μm) Lake Michigan water (150 ml) were added to the jars, and the slurry was rolled for 1.5 h at 15°C, held overnight at 4°C, and rolled again at 15°C for 5 h. Spiked sediments for the first experiment were shipped back to the GLERL on ice. Sediments for both

experiments were held to equilibrate at 4°C for 60 d prior to the start of the experiment to allow for dissolution and partitioning of spiked fluoranthene to occur.

Immediately after spiking and mixing in the first experiment, the concentration of [^{14}C]fluoranthene was determined by combustion of sediment in a sample oxidizer (Model 306A, Packard Instrument Co., Downers Grove, IL, USA) and quantified by LSC. In the first experiment, [^{14}C]fluoranthene was also measured immediately after dosing and on exposure days 0, 4, 10, 17, and 30 by sonication (1 min) of sediment samples (50–100 mg) in scintillation cocktail 3a70b, Research Products International, Mt. Prospect, IL, USA) with a Tekmar (Cincinnati, OH, USA) high-intensity probe-sonicator (375 W at 20% power), followed by LSC. In the second experiment, sediment samples (50–100 mg) taken on days 0, 4, 10, 16, and 30 were held overnight in scintillation cocktail, without sonication, prior to LSC. Direct comparisons showed no significant difference between activity of samples before and after sonication in the second experiment (unpublished results).

Exposure

Diporeia were collected in December 1994 by ponar grab from Lake Michigan off Grand Haven, Michigan, USA, at a depth of 24 to 29 m, and held at 4°C in native sediment and unfiltered Lake Michigan water until the start of the experiments. *Diporeia* from this site have previously been shown to contain low body burdens of PAHs [26]. *Hyalella azteca* were obtained from Chris Ingersoll at the National Biological Service in Columbia, Missouri, USA in December 1994 and June 1995. *Hyalella azteca* were of a size that passed through a 1-mm sieve, but were retained on a 500-μm sieve (approximately 2–3 weeks old). Animals were gradually acclimated to local water before the start of the experiment. Filtered water (0.45 μm) from the nearby Huron River, which closely matches Lake Michigan water in terms of hardness, alkalinity, and pH (see Results), was used during exposures.

At the end of the 60-d equilibration period, spiked sediment was stirred to visual homogeneity. Sediment (100 g wet weight) was added to each exposure beaker (300 ml tall form) prior to the careful addition of overlying water (250 ml). Sediment was allowed to settle for 1 d prior to the addition of animals. Ten animals were added per beaker on day 0. Beakers were randomly placed into water renewal systems [28]. In order to maintain sufficient levels of dissolved oxygen and reduce ammonia in the beakers, approximately one third of the overlying water was exchanged twice per day. Water quality characteristics, including hardness, alkalinity, pH, temperature, dissolved oxygen, ammonia, and radioactivity were measured at various time points throughout the experiment. Hardness and alkalinity were measured with commercially available kits (CHEMetrics, Calverton, VA, USA) by standard titration methods. Oxygen was measured with an oxygen electrode (Orion Research, Boston, MA, USA). Ammonia was determined by an automated colorimetric phenate method [29] on a Technicon Auto Analyzer II (Technicon Instruments Corp., Tarrytown, NY, USA). Subsamples of overlying water (2 ml) were analyzed by LSC for concentration of radiolabeled fluoranthene. *Hyalella azteca* were fed every day with 1.0 ml of yeast–cerophyl–trout chow (YCT) per beaker according to EPA guidelines [21]. *Diporeia* were not fed, according to ASTM guidelines [30]. Experiments were run at 4°C for *Diporeia* and at room temperature for *H. azteca*.

At each sampling period, surface sediment (<2 cm) was sieved (500 μm) and the number of live and dead animals found was recorded. Death was defined by the absence of movement upon examination with a dissecting microscope. Subsurface sediment samples for fluoranthene concentration and wet-to-dry weight determinations were taken carefully, excluding any organisms, from each beaker sampled. Remaining sediment was then sieved for additional animals. Percent survival was calculated on the basis of the number of live animals recovered divided by the total number of animals added. Missing animals were counted as dead. After determination of wet weights, live animals from the first experiment were transferred into scintillation cocktail, probe-sonicated (1 min), and analyzed by LSC. In the second experiment, live animals were held in scintillation cocktail overnight, without sonication, prior to LSC. At the end of the first experiment, and at the beginning and end of the second experiment, the lipid content of some animals was determined using a microgravimetric technique [31]. Growth rates of both species were calculated from the regression of the natural log of wet weight versus exposure time.

Sediment

At the end of the experiments, sediment samples were taken from several concentrations (five from each experiment) and extracted to determine the fluoranthene concentration by GC/MS [22]. After the addition of deuterated surrogate PAH standards and acetone (75 ml), sediment samples (1 g wet weight) were sonicated (60 min at 30°C). Methylene chloride (50 ml) was added, and samples were sonicated (60 min at 30°C), incubated overnight (30°C), and sonicated again for 1 h. Extracts for GC/MS were filtered over glass wool prior to extraction with distilled water (400 ml), and NaCl-saturated water (50 ml). The aqueous phase was reextracted with methylene chloride (40 ml), organic phases were combined and evaporated to 5 ml, and hexane (15 ml) was added. Extracts were prepared for GC/MS by chromatography on a silica gel column. Columns were eluted with hexane (50 ml), followed by hexane/methylene chloride (60:40, v/v). Samples were reduced to 1 ml, and internal PAH standards were added prior to analysis by GC/MS [22].

Sediment samples, taken from every concentration at the end of the experiments, were extracted to determine the percent purity of the fluoranthene by thin-layer chromatography (TLC). Sediment samples were dried with anhydrous sodium sulfate, extracted with methylene chloride and acetone as described above, evaporated to 0.5 ml, and run on silica gel plates in benzene/hexane (20:80, v/v). Radioactivity was quantified by LSC.

Organic carbon content of the sediment was analyzed on a model 2400 CHN Elemental Analyzer (Perkin Elmer Corp., Norwalk, CT, USA) after acidification to remove carbonates. Triplicate samples, taken on days 0 and 30, were analyzed for each sediment concentration.

Pore water

For the first experiment, sediment samples (50 g wet weight) taken on day 4 from beakers that had contained animals were centrifuged (30 min at 20,000 *g*) to obtain pore water. For the second experiment, larger sediment samples (150 g wet weight) were taken so that the pore water could be analyzed for dissolved organic carbon. Sediment samples for experiment 2, taken on day 10 from beakers that had not contained animals, were centrifuged at low speed (30 min at

1,200 *g*) to pellet the sediment, followed by a high speed spin (30 min at 20,000 *g*) to collect the pore water and remove larger colloids from the pore water. Radioactivity associated with the supernatant was determined by LSC. Aliquots of the remaining supernatants were passed through C18 Sep-Pak cartridges (Millipore Corp., Bedford, MA, USA) using a reverse-phase separation method for determining the binding of compound to dissolved organic carbon in the pore water [32]. Radiolabeled compound that passed through the column and was presumably complexed with dissolved organic carbon was measured by LSC. In the second experiment, the radioactivity that bound to the Sep-Pak, presumably representing freely dissolved fluoranthene, was eluted with methanol and quantified by LSC. In the second experiment, the concentration of dissolved organic carbon in the centrifuged supernatant was measured on a Shimadzu Total Organic Carbon Analyzer (TOC-5000, Shimadzu Corp., Kyoto, Japan).

Complexation of compound to Huron River water that was collected for experiment 1, but not used in the experiment, was also analyzed. Filtered water was dosed with 774 ng/L [^{14}C]fluoranthene, stirred for 2 h, and passed through a Sep-Pak cartridge. The radioactivity that passed through the Sep-Pak without binding was quantified by LSC.

Statistics

Linear and nonlinear regression and probit analyses were performed with *SAS®/STAT, Version 6*, 4th edition (SAS Institute, Cary, NC, USA). Mortality data were also analyzed with the trimmed Spearman–Karber method using *Statistical Methods and Software for Toxicological Data Analysis* (B.A. Zajdlik, University of Waterloo, Waterloo, ON, Canada, and M.A. Newman, Savannah River Ecology Lab, Aiken, SC, USA). Student's *t* test was used when comparing percent survival (arcsine-transformed data), means, or slopes of regression lines. Differences (1-tailed *t* tests) were considered significant when $p < 0.05$. Confidence limits for LD50s were determined according to a recommended method [33].

Modeling

Accumulation of fluoranthene from sediment was modeled using a previously described general model [34]:

$$dC_a/dt = k_s C_s^0 e^{-\lambda t} - k_d C_a \quad (1)$$

where C_a is the concentration in the animal (nmol/g wet weight), k_s is the conditional uptake clearance rate coefficient (g dry sediment/g wet weight organism/d), C_s^0 is the initial concentration in the sediment (nmol/g dry sediment), λ is the conditional rate constant (d^{-1}) for reduction in the bioavailable fraction of fluoranthene, k_d is the conditional elimination rate constant (d^{-1}), and t is time (d). For these experiments, uptake clearance rates (k_s) for *Diporeia* were fit by nonlinear regression to the integrated form of the general model:

$$C_a = \frac{(k_s C_s^0)(e^{-\lambda t} - e^{-k_d t})}{(k_d - \lambda)} \quad (2)$$

An average value for k_d of 0.0648 d^{-1}, determined for the elimination of fluoranthene by *Diporeia* in the presence of sediment [35], was used in modeling *Diporeia* data from these experiments.

RESULTS

Test conditions

Dissolved oxygen levels measured 6 h after exchange of water on days 1 and 25 for the first experiment and on day 8 for the second experiment averaged 75.9% (n = 30, CV = 6%) and 67.2% (n = 15, CV = 3%) saturation for *Diporeia*, and 65.6% (n = 43, CV = 13%) and 59.2% (n = 34, CV = 12%) saturation for *H. azteca*, in experiments 1 and 2, respectively. Ammonia levels measured on day 10 averaged 65.4 μg N/L as NH_3 (n = 30, CV = 21%) and 26.1 μg/L (n = 6, CV = 54%) for *H. azteca*, and 186.8 μg N/L (n = 30, CV = 18%) and 155.0 μg/L (n = 6, CV = 26%) for *Diporeia*, in experiments 1 and 2, respectively. Average alkalinity (mg/L total alkalinity as calcium carbonate) was 250 mg/L (n = 48) and 270 (n = 15) for *Diporeia*, and 230 (n = 48) and 260 (n = 15) for *H. azteca*, in experiments 1 and 2, respectively. Average hardness (mg/L total hardness as calcium carbonate) was 160 (n = 22) and 175 (n = 15) mg/L for *Diporeia*, and 160 (n = 22) and 170 (n = 15) mg/L for *H. azteca*, in experiments 1 and 2, respectively. Average pH was 8.4 (n = 30, CV = 0.3%) and 8.1 (n = 30, CV = 0.5%) for *Diporeia* and *H. azteca*, respectively, in the first experiment. Average temperatures were 4.8°C (n = 30, CV = 1.3%) and 4.7°C (n = 18, CV = 5%) for *Diporeia* and 20°C (n = 30, CV = 0.2%) and 24.0°C (n = 36, CV = 1.7%) for *H. azteca*, in experiments 1 and 2, respectively. The higher temperature for *H. azteca* in the second experiment reflected a higher room temperature during the summer of 1995. No significant treatment effects were observed for water quality characteristics. The concentration of radiolabeled fluoranthene in 2-ml subsamples of overlying water was not significantly different from background.

Sediment

In most cases, the sediment fluoranthene concentration (as measured by sonication/LSC) at day 30 declined slightly (range = 2.7–16.3%) compared to the concentration measured on day 0 (Table 1). However, measured concentrations did not decline with time in all cases. Therefore, all LSC measurements taken over the course of the exposure period (days 0, 4, 10, 16 or 17, and 30) were averaged for each nominal concentration (Table 1). Percent recovery of spiked compound, made by comparison of these overall average values to nominal concentrations, ranged from 86.3% at the lowest concentration (excluding trace level) to 54.2% at a higher concentration for the first experiment, and from 80.6 to 69.0% in the second experiment. As expected, measured concentrations were generally lower than nominal concentrations, presumably due to loss of compound during spiking. Direct comparison of the sonication method versus the oxidation method showed that, on average, the oxidation values were 102 ± 13% (n = 33) of the sonication values for samples taken immediately after dosing and were not significantly different.

Except for trace levels, the concentrations of fluoranthene measured by sonication/LSC (day 30) were within a factor of two of the concentrations measured by GC/MS at the end of the experiment (day 30) (Table 2). At the end of the first experiment, sediment fluoranthene was found to be on average 86.2% pure parent compound (range = 83.0–96.3%, Table 2), based on TLC and radiometric analysis. At the end of the second experiment, sediment fluoranthene was on average 92.5% parent compound for *Diporeia* and 89.3% for *H. azteca* exposures (Table 2).

In the first experiment, average percent organic carbon was 0.38% (day 0, n = 15, CV = 9%) and 0.35% (day 30, n = 18, CV = 18%) for *Diporeia* and 0.35% (day 0, n = 15, CV = 9%) and 0.36% (day 30, n = 18, CV = 11%) for *H. azteca*.

Table 1. Concentration of fluoranthene in sediment samples taken at the beginning and end of the exposures

Organism	Nominal sediment concn. (nmol/g dry wt.)	Experiment 1: Mean (SD) measured sediment concn., day 0 (nmol/g dry wt.)	Experiment 1: Mean (SD) measured sediment concn., day 30 (nmol/g dry wt.)	Experiment 1: Overall mean (SD) (*n*) measured sediment concn. (nmol/g dry wt.)	Experiment 2: Mean (SD) measured sediment concn., day 0 (nmol/g dry wt.)	Experiment 2: Mean (SD) measured sediment concn., day 30 (nmol/g dry wt.)	Experiment 2: Overall mean (SD) (*n*) measured sediment concn. (nmol/g dry wt.)
Diporeia	0.1	0.102 (0.015)	0.096 (0.002)	0.1 (0.01) (19)	0.118 (0.001)	0.113 (0.015)	0.114 (0.008) (17)
	160	75 (4)	73 (5)	77 (6) (16)	141 (4)	143 (19)	138 (9) (13)
	320	241 (29)	214 (51)	242 (45) (15)	206 (7)	216 (11)	190 (37) (17)
	630	394 (45)	344 (76)	370 (64) (16)	341 (20)	364 (101)	340 (96) (14)
	1,270	676 (36)	687 (83)	688 (99) (18)	657 (88)	542 (38)	769 (429) (17)
Hyalella azteca	0.1	0.108 (0.006)	0.090 (0.007)	0.1 (0.01) (18)	0.124 (0.002)	0.091 (0.021)	0.110 (0.014) (16)
	40	49 (7)	41 (0.67)	44 (5) (14)	ND[a]	ND	ND
	80	84 (7)	73 (11)	74 (10) (15)	ND	ND	ND
	160	85 (9)	81 (4)	85 (7) (14)	130 (5)	114 (34)	129 (19) (14)
	320	205 (47)	192 (51)	136 (71) (17)	275 (11)	251 (15)	257 (31) (17)
	630	ND	ND	ND	426 (82)	395 (41)	392 (65) (14)
	1,270	ND	ND	ND	975 (82)	621 (190)	876 (328) (17)

[a] ND not determined.

In the second experiment, average percent organic carbon was 0.53% (day 0, n = 15, CV = 32%) and 0.49% (day 30, n = 16, CV = 17%) for *Diporeia* and 0.47% (day 0, n = 14, CV = 20%) and 0.53% (day 30, n = 15, CV = 20%) for *H. azteca*.

In both experiments, measures of pore-water concentration were in general agreement with predicted values (Table 3). The percent of total compound that was freely dissolved was estimated by difference from the percent that was complexed to organic matter and passed through the Sep-Pak C18 cartridges. Percent of total compound that was freely dissolved ranged from about 9% for the trace concentrations, to about 30 to 70% at higher sediment concentrations. In contrast, an average of only 2.3% (SD = 0.4%, n = 4) of [^{14}C]fluoranthene dosed to unused, filtered Huron River water passed through a Sep-Pak, resulting in an estimated 97.7% freely dissolved compound. A mass balance for the second experiment indicated that an average of 54% (SD = 18%, n = 10) of the total radioactivity that was passed through the Sep-Pak was recovered as the sum of radioactivity that flowed through the Sep-Pak or was subsequently eluted with methanol. Incomplete recovery may be due in part to incomplete elution of compound from the Sep-Pak.

Mortality

In the first experiment, survival of *Diporeia* after 10 d exhibited a general dose response, ranging from 90% survival in the control treatment (without fluoranthene) to 62% survival at the highest sediment concentration (688 nmol/g dry weight) (Table 4). Percent survival was significantly reduced in two out of three of the highest concentrations (242 and 370 nmol/g dry weight) compared to controls. Probit and other analyses of the *Diporeia* 10-d mortality data were unsuccessful, presumably because 50% mortality was not attained. After 17 and 30 d of exposure, survival in *Diporeia* was reduced to as low as 40% and 14% at the highest concentrations (Table 4). After 30 d, survival was significantly reduced in comparison to controls in the four highest sediment concentrations. Because control mortality for days 17 and 30 exceeded that required for a successful test (10% maximum allowable control mortality, based on the 1995 ASTM guideline for *Diporeia*) [29], LC50s

Table 2. Percent purity and concentration of fluoranthene in sediment at end of experiments[a]

Organism	Experiment 1, day 30: Mean measured sediment concn., LSC (n = 5) (nmol/g dry wt.)	Experiment 1, day 30: Measured sediment concn., GC/MS (n = 1) (nmol/g dry wt.)	Experiment 1, day 30: Mean % purity (n = 2)	Experiment 2, day 30: Mean measured sediment concn., LSC (n = 5) (nmol/g dry wt.)	Experiment 2, day 30: Measured sediment concn., GC/MS (n = 1) (nmol/g dry wt.)	Experiment 2, day 30: Mean % purity (n = 2)
Diporeia	0.096	3	87.2	0.113	ND	81.4
	73	95	83	143	83.7	96.0
	214	ND	91.4	216	200.5	93.7
	344	605	91.8	364	256.4	95.4
	687	1,003	89.0	542	ND	96.1
Hyallela azteca	0.090	ND	90.5	0.091	ND	80.7
	41	ND	95.5	114	90.5	81.8
	73	ND	91.2	251	ND	93.3
	81	109	96.3	395	334	94.7
	192	ND	86.0	621	ND	96.0

[a] LSC liquid scintillation counting, GC/MS gas chromatography/mass spectrometry, ND = not determined.

Table 3. Estimated and measured interstitial water concentration and percent freely dissolved fluoranthene

Organism	Experiment 1				Experiment 2				
	Mean measured sediment concn. (μmol/g organic carbon)	Estimated[a] interstitial water concn. (nmol/L)	Mean (SD) measured total interstitial water concn. (nmol/L) (n = 3)	Mean (SD) measured % freely dissolved (n = 3)	Mean measured sediment concn. (μmol/g organic carbon)	Estimated[a] interstitial water concn. (nmol/L)	Measured total interstitial water concn. (nmol/L) (n = 1)	Interstitial water dissolved organic carbon (mg/L) (n = 1)	Measured % freely dissolved (n = 1)
Diporeia	0.027	0.27	0.237 (0.04)	9 (8.0)	0.022	0.22	0.089	27.5	8.9
	20.8	208	282 (7.4)	45 (10.5)	26.0	260	289	27.0	69
	65.4	654	732 (26.2)	49 (2.1)	35.8	358	347	26.7	64
	100	1,000	1,070 (17.8)	44 (11.9)	64.2	642	386	59.3	56
	186	1,860	1,760 (74.1)	33 (4.4)	145	1,450	595	28.9	30
Hyalella azteca	0.027	0.27	ND[b]	ND	0.021	0.21	0.016	31.7	42
	11.9	119	ND	ND	24.3	243	230	31.4	71
	20.0	200	ND	ND	48.5	485	401	32.1	59
	23.0	230	ND	ND	74.0	740	549	35.8	52
	36.8	368	ND	ND	165	1,650	569	42.3	60

[a] Calculations used a K_{ow} value of 10^5, average organic carbon content of 0.37% for experiment 1 and 0.53% for experiment 2, and measured sediment concentrations.
[b] ND = not determined.

were not estimated for these time points. In the second experiment, less mortality was observed in *Diporeia* than was found in the first experiment. However, percent survival was significantly reduced after 30 d of exposure to the four highest sediment concentrations (Table 4). Probit and trimmed Spearman–Karber analyses of mortality data for *Diporeia* in the second experiment were unsuccessful.

In both experiments, average survival by *H. azteca* after 10- or 16-d exposures was greater than 90% at all concentrations and was not significantly different from controls (Table 4). After a 30-d exposure in the first experiment, survival was still high, greater than 96%. After a 30-d exposure in the second experiment, survival was significantly reduced at two concentrations, but still greater than 74% at all concentrations (Table 4). Missing *H. azteca* were counted as dead because recent studies have demonstrated that dead *H. azteca* decompose rapidly (within 12 h) in sediments [36].

Bioaccumulation

In the first experiment, comparison between species of the accumulation of fluoranthene from sediments spiked at nominal concentrations of 0.1, 160, and 320 nmol/g dry weight (the only concentrations in the first experiment to which both species were exposed) shows that *H. azteca* accumulated more compound than did *Diporeia* over the first 1 to 2 d of exposure (Fig. 1a to c). Thereafter, the body burden of *H. azteca* declined, whereas the body burden of *Diporeia* continued to increase until reaching an apparent steady state after 10 d.

Table 4. Mean (SD) percent survival calculated as (number of live animals recovered/number of animals exposed)·100. Missing animals were counted as dead. Because control samples were not taken on day 17, trace concentrations (0.1 nmol/g dry wt.) serve as controls for mortality at that time point

Organism	Experiment 1				Experiment 2			
	Mean measured sediment concn. (nmol/g dry wt.)	Day 10 (n = 5)	Day 17 (n = 3)	Day 30 (n = 5)	Mean measured sediment concn. (nmol/g dry wt.)	Day 10 (n = 5)	Day 16 (n = 3)	Day 30 (n = 5)
Diporeia	Control	90 (10)	ND[a]	68 (13)	Control	81 (16)	ND	98 (4)
	0.1	94 (13)	70 (35)	67 (17)	0.1	90 (12)	100 (0)	96 (5)
	77	84 (13)	73 (12)	28 (13)*[b]	138	90 (10)	97 (6)	86 (9)*
	242	68 (18)*	60 (0)	24 (19)*	190	86 (9)	97 (6)	63 (36)*
	370	70 (10)*	37 (35)	20 (34)*	340	90 (0)	93 (6)	80 (16)*
	688	62 (27)	40 (17)	14 (21)*	769	86 (5)	73 (25)	53 (36)*
Hyalella azteca	Control	96 (5)	ND	100 (0)	Control	98 (4)	ND	94 (5)
	0.1	96 (9)	97 (6)	98 (4)	0.1	98 (4)	100 (0)	86 (5)*
	44	100 (0)	100 (0)	100 (0)	129	98 (4)	100 (0)	96 (5)
	74	92 (8)	100 (0)	96 (5)	257	96 (9)	97 (6)	92 (4)
	85	90 (7)	97 (6)	100 (0)	392	94 (5)	100 (0)	78 (22)
	136	98 (4)	97 (6)	96 (5)	876	90 (10)	97 (6)	74 (13)*

[a] ND = not determined.
[b] * = significantly different from control ($p < 0.05$).

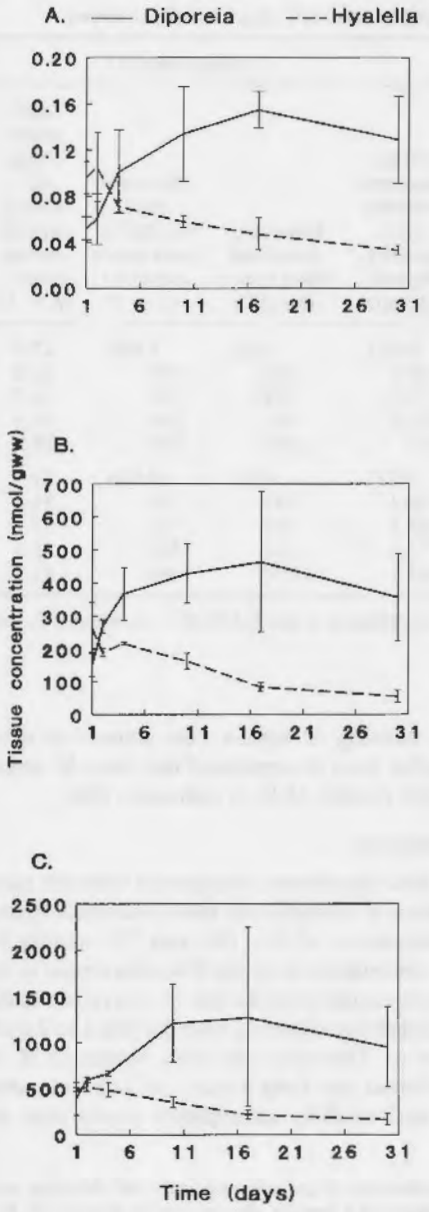

Fig. 1. Accumulation of fluoranthene in tissue of *Diporeia* and *Hyalella azteca* over time in experiment 1, after exposure to nominal fluoranthene sediment concentrations of (A) 0.1 nmol/g dry weight, (B) 160 nmol/g dry weight, and (C) 320 nmol/g dry weight. Error bars represent standard deviations of three to five samples.

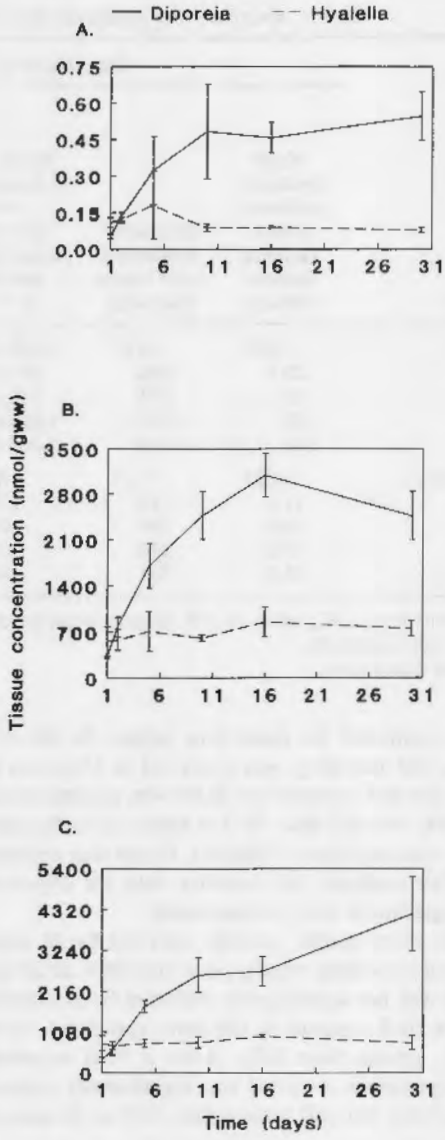

Fig. 2. Accumulation of fluoranthene in tissue of *Diporeia* and *Hyalella azteca* over time in experiment 2, after exposure to nominal fluoranthene sediment concentrations of (A) 0.1 nmol/g dry weight, (B) 630 nmol/g dry weight, and (C) 1,270 nmol/g dry weight. Error bars represent standard deviations of three to five samples.

Similar accumulation kinetics for the two species were observed at other sediment concentrations (data not shown). At the highest sediment concentrations (370 and 688 nmol/g wet weight, measured concentrations), observed body burdens in *Diporeia* were in the range of 1 to 2 μmol/g wet weight tissue after 10 d, a dose that could be expected to result in death by narcosis. The body burden of *H. azteca,* in general, did not exceed 0.5 μmol/g wet weight, which is consistent with the absence of observed mortality (Fig. 1). A similar pattern of accumulation was observed in the second experiment. Typical uptake curves are shown for accumulation of fluoranthene from nominal sediment concentrations of 0.1, 630, and 1270 nmol/g dry weight (Fig. 2). After 10 d of exposure to the highest sediment concentrations (Fig. 2b and c), *Diporeia* tissue concentrations were in the range of 2 to 4 μmol/g wet weight tissue, but *H. azteca* did not, in general, accumulate more than 1 μmol total fluoranthene equivalents/g ww.

The relationship between percent survival of *Diporeia* versns the tissue concentration of surviving amphipods (taken from all sediment concentrations) was used to estimate critical body burden. In the first experiment, a significant linear regression was found for the 10-d exposure (Fig. 3a), but not for the 17-d (Fig. 3b) or 30-d exposures (not shown). From the relationship for the 10-d exposures, an estimated tissue concentration of 2,700 (930–12,900, 95% CI) nmol fluoranthene/g wet weight would be associated with 50% mortality. Because 50% mortality was not actually achieved after 10-d exposures in these experiments, this LD50 should only be considered a rough estimate. If the outlier data point associated with trace exposure and 30% survival is removed from the 17-d data set (Fig. 3b), the regression is significant ($p = 0.009$, $r = 0.689$) and 50% mortality is associated with a body burden of 2,252 nmol/g wet weight. Factors other than exposure to

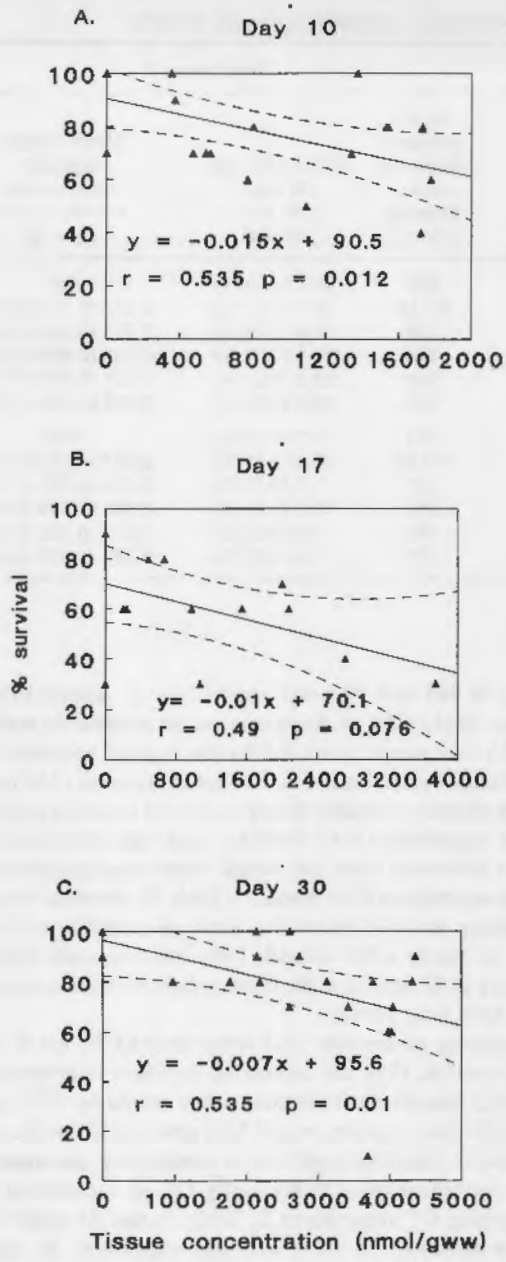

Fig. 3. Concentration of fluoranthene in tissue versus percent survival for *Diporeia*, after (A) 10 d of exposure in experiment 1, (B) 17 d of exposure in experiment 1, or (C) 30 d of exposure in experiment 2, to a range of sediment fluoranthene concentrations. Dashed lines represent 95% confidence intervals.

fluoranthene were probably contributing to the mortality observed at 30 d, as the percent survival in the unexposed, control animals (68%) and animals exposed to trace concentrations (67%) were substantially lower on day 30 than the percent survival observed in animals exposed to control sediments (90%) or trace concentrations (94%) on day 10 (Table 4). In the second experiment, a significant relationship for percent survival versus tissue concentration was observed for *Diporeia* after a 30-d exposure (Fig. 3c) but not at the earlier time points. On the basis of the 30-d exposure data, the estimated body burden associated with 50% mortality would be 6,500 (3,400–25,280, 95% CI) nmol/g wet weight. Note that other relationships, such as regression of log-transformed tissue concentrations, were examined, but did not provide a better fit to the data (data not shown).

In the first experiment, lipid contents (dry weight basis) of tissue samples taken at the end of the experiment suggest a dose-dependent decrease in lipid content in *Diporeia*, but not in *H. azteca* (Table 5). Lipid contents of *Diporeia* that were exposed to trace levels of fluoranthene were not significantly different from those of unexposed, control animals, but average lipid contents from animals that were exposed to 77 or 242 nmol/g dry weight were significantly lower than controls. Only two samples of *Diporeia* exposed to the highest sediment concentration were analyzed, and the average lipid content was not significantly different from controls.

In the second experiment, lipid content of unexposed organisms sampled at the beginning of the experiment (day 0) was 21.4% for *Diporeia* (n = 2, CV = 26%) and 7.4% for *Hyalella* (n = 4, CV = 23%). Lipid content of *Diporeia* at the end of the experiment ranged from 19.9 to 24.1% and lipid contents of animals exposed to fluoranthene were not significantly different from those of controls (Table 5). Lipid content of *Hyalella* on day 30 ranged from 6.3 to 10.2% (Table 5).

Apparent steady state BSAFs were calculated for *Diporeia* and *Hyalella* using measured 17-d (or 16-d for experiment 2) sediment and tissue concentrations, dose-specific values for lipid content, and overall average measured sediment organic carbon (OC) values (0.0037 g OC/g dry weight sediment for the first experiment and 0.0053 g OC/g dry weight for the second experiment)(Table 5). In the first experiment, an average value of 9.3% lipid was used for *Diporeia* exposed to 370 nmol/g dry weight, because no lipid data was available. Literature values (0.27) for the dry to wet weight ratio of both *Diporeia* [34] and *H. azteca* [37] were used to convert tissue concentrations to dry weights. In the first experiment, mean BSAFs (n = 3) were substantially lower for both species exposed to trace concentrations of fluoranthene compared to higher sediment concentrations. Maximum BSAFs were observed at intermediate concentrations in all cases. The BSAFs for *H. azteca* were generally lower than those of *Diporeia*. Because BSAFs presented here are based on concentrations of total fluoranthene equivalents (parent compound and metabolites) and because *H. azteca* is known to have a greater ability to metabolize fluoranthene than does *Diporeia* [35], BSAFs for *H. azteca* based on parent compound alone could be even lower.

In both experiments, calculated uptake rate coefficients were maximum at intermediate sediment concentrations (1.307 and 1.37 g dry weight sediment/g wet weight organism/d for experiments 1 and 2, respectively) and smaller for animals exposed to trace doses or maximum sediment concentrations (0.276–0.731 g dry weight sediment/g wet weight organism/d) (Table 6). Values for λ, the rate constant for the reduction in the bioavailable fraction of fluoranthene in the sediment, were higher in the first experiment (0.052–0.100/d) than in the second experiment (0.004–0.033/d). Values of λ in the second experiment were lower than values previously measured for the accumulation of phenanthrene by *Diporeia* (0.079–0.192/d) [13]. This parameter was originally conceived to describe the rate at which a contaminant moves to a biologically unavailable pool [34] and was presumed to reflect changes in chemical extractability and the reversible/slowly reversible partitioning of contaminants in sediments [38]. An alternative explanation for the decline in body burden observed in the present experiments and in other 30-d sediment exposures [34]

Table 5. Percent lipid content and apparent steady-state biota–sediment accumulation factors (BSAFs)

Organism	Experiment 1			Experiment 2		
	Mean measured sediment concn. (nmol/g dry wt.)	Mean (SD) (*n*) % lipid (dry wt.), day 30	Mean (range) apparent steady-state BSAF, day 17 (*n* = 3)	Mean measured sediment concn. (nmol/g dry wt.)	Mean (SD) (*n*) % lipid (dry wt.), day 30	Mean (range) apparent steady-state BSAF, day 16 (*n* = 3)
Diporeia	Control	21.3 (6.7) (5)	ND[a]	ND	20.1 (4.6) (5)	ND
	0.1	15.2 (7.7) (3)	0.107 (0.097–0.119)	0.114	20.6 (0.8) (2)	0.436 (0.372–0.492)
	77	11.0 (3.5)*[b] (3)	0.747 (0.389–1.069)	138	21.4 (3.8) (4)	0.778 (0.687–0.944)
	242	10.3 (3.5)* (4)	0.649 (0.102 -1.057)	190	20.1 (1.9) (3)	0.818 (0.696–0.942)
	370	ND	0.697 (0.365–0.993)	340	23.3 (4.2) (4)	0.766 (0.695–0.857)
	688	8.3 (7.7) (2)	0.424 (0.214 0.821)	769	19.9 (3.6) (3)	0.345 (0.290 0.384)
Hyalella azteca	Control	8.4 (0.7) (5)	ND	ND	8.2 (0.7) (4)	ND
	0.1	10.2 (1.0)* (5)	0.045 (0.034–0.060)	0.110	8.6 (1.8) (3)	0.231 (0.192-0.275)
	44	7.3 (2.1) (4)	0.329 (0.237 0.389)	129	7.2 (3.2) (3)	0.300 (0.293 0.312)
	74	6.3 (2.0) (5)	0.274 (0.229–0.307)	257	8.3 (1.4) (4)	0.398 (0.359–0.487)
	85	7.2 (3.6) (4)	0.161 (0.146 0.188)	392	7.0 (2.9) (3)	0.612 (0.458 0.784)
	136	6.4 (1.2) (5)	0.236 (0.187–0.290)	876	9.3 (0.3) (3)	0.234 (0.222–0.242)

[a] ND = not determined.
[b] * = significantly different from control ($p < 0.05$).

might include changes in the physiology of the organisms (such as narcosis) that result in a reduction in the rate of accumulation of contaminant [35].

Uptake of fluoranthene by *H. azteca* did not fit any available models, including growth dilution models; therefore, uptake rates are not presented for this species. *Diporeia* showed no significant growth over the course of the experiment, but *H. azteca* exhibited growth at all doses (Table 7). There was no apparent dose-dependent effect on growth.

DISCUSSION

The EqP approach predicts that sediment toxicity can be predicted from effects determined in water-only exposures. Interstitial water concentrations in these experiments (Table 3) were estimated to overlap the range of reported *H. azteca* 10-d water-only LC50s for fluoranthene of 221 nmol/L [24] and 299.6 nmol/L (B. Suedel, personal communication), as well as the range of acute values (178–1,046 nmol/L, 96-h LC50 or median effective concentration [EC50]) measured in water-only tests with 13 freshwater species, and the final acute value (FAV = 166 nmol/L) and final chronic value (FCV = 30.5 nmol/L) derived from those tests [23]. In addition, two water-only experiments performed in our laboratory produced 10-d LC50s of 481 and 564 and nmol/L for *H. azteca* [35]. The average 10-d LC50 of these two recent water-only tests (522 nmol/L) was nearly exceeded by the highest estimated interstitial water concentration in the first experiment (366 nmol/L) and the highest estimated freely dissolved concentration in the second experiment (341 nmol/L), and was exceeded by the highest measured total interstitial water concentration in the second experiment (569 nmol/L) (Table 3). Because these concentrations are well below the limit of solubility of fluoranthene in water, 1,285 nmol/L [39], we expected significant mortality in *H. azteca* at the highest concentrations, especially at the later time points.

Assuming an average 10-d water-only LC50 for *H. azteca* of 522 nmol/L [35], the calculated sediment concentration to yield 522 nmol/L in interstitial water would be 52.2 μmol/g OC for the first experiment and 52.3 μmol/g OC for the second experiment. Based on equilibrium partitioning, maximum sediment concentrations (36.8 μmol/g OC in experiment 1 and 165 μmol/g OC experiment 2, Table 3) should result in significant mortality in 10-d sediment exposures. In addition, nominal concentrations of fluoranthene in sediment in the first experiment (40–320 nmol/g dry weight) were chosen to bracket the range of published LC50 and EC50 (immobility) values

Table 6. Uptake rate coefficients (k_u) for *Diporeia* and rate constants (λ) for the reduction in the bioavailable fraction of fluoranthene

Experiment	Mean measured sediment concn. (nmol/g dry wt.)	k_u (g dry wt. sediment/g wet wt. tissue/d)	95% Confidence interval		λ (d^{-1})
			Lower	Upper	
1	0.1	0.276	0.206	0.347	0.062
	77	1.37	0.953	1.781	0.089
	242	1.03	0.578	1.483	0.075
	370	0.951	0.511	1.392	0.100
	688	0.476	0.241	0.712	0.052
2	0.1	0.731	0.523	0.948	0.033
	138	1.307	0.868	1.746	0.016
	190	1.241	0.908	1.574	0.004
	340	1.054	0.877	1.231	0.011
	769	0.434	0.334	0.534	0.004

Table 7. Growth rate of *Hyalella azteca*, calculated from the regression of ln(wet wt.) versus exposure time

Experiment	Mean measured sediment concn. (nmol/g dry wt.)	Growth rate constant (d^{-1})	Standard error	r^2
1	0.1	0.067	0.004	0.935
	44	0.056	0.004	0.905
	74	0.068	0.007	0.833
	85	0.058	0.002	0.970
	136	0.056	0.006	0.816
2	0.1	0.106	0.017	0.732
	129	0.105	0.010	0.888
	257	0.084	0.016	0.636
	392	0.074	0.010	0.771
	876	0.115	0.023	0.630

for this species, 11.4 to 36.6 nmol/g dry weight (EC50) [24], 76.1 nmol/g dry weight (LC50) [25], and 150 nmol/g dry weight (LC50, B. Suedel, personal communication). Although measured sediment concentrations in the second experiment were even higher (up to 876 nmol/g dry weight), minimal mortality was observed.

Hyalella azteca did not attain the dose of fluoranthene required for mortality, and its body burden actually declined over time (Figs. 1 and 2). We hypothesize that the behavior of *Hyalella* in this test contributed to its low body burden. Although the animals initially burrowed into the sediment, after day 5 almost all of the animals remained on the surface and did not burrow into the sediment. Behavioral avoidance of sediment dosed with high concentrations of dieldrin (1,000–5,000 μg/g OC) was proposed as an explanation for lower than predicted mortality in *H. azteca* in a previous experiment [40]. In the present experiments, however, animals at all concentrations (including controls) appeared to remain on the surface, where they were exposed to overlying water that did not contain measurable concentrations of fluoranthene. This behavior may explain the initial increase and subsequent decline in body burden that was observed in this experiment.

Alternatively, the low observed body burden might be explained by the biotransformation capability of *H. azteca*. *Hyalella azteca* is known to biotransform PAHs, specifically anthracene [37] and fluoranthene [35]. Further, after water-only exposures to various concentrations of fluoranthene, observed elimination half-lives were 3 to 6 h for *H. azteca*, and 7 to 25 d for *Diporeia* [35]. Thus, *H. azteca* might eliminate a substantial fraction of its total body burden when exposed to uncontaminated overlying water.

Finally, *H. azteca* were fed uncontaminated food throughout the exposure. Such feeding, although a requirement for sediment testing with this organism, leads to both organism growth (which can dilute tissue concentrations) and perhaps reduced exposure. In a separate experiment, the influence of this feeding was eliminated as the likely cause of the reduced exposure to fluoranthene, because feeding was shown to increase uptake of fluoranthene by *Hyalella* [41].

To estimate the sediment concentration that would result in significant mortality in *Diporeia* within 10 d, toxicokinetic calculations were performed using the following equation:

$$C_a = (k_u/k_d)(C_{sed})(1 - e^{-k_d t}) \quad (3)$$

where C_a is an estimated lethal concentration in the animal (6 mmol/kg tissue), C_{sed} is the sediment concentration, and t is time (10 d). The conditional uptake clearance coefficient (k_u = 0.432 g dry sediment/g wet weight organism/d) and elimination rate constant (k_d = 0.048/d) used in these preliminary calculations were previously determined for *Diporeia* with pyrene (with a K_{ow} of 5.2, similar to that of fluoranthene) [13], but were found to overlap the range of uptake rate coefficients measured in the present experiments (Table 6) and elimination rate coefficients measured in a related study (0.026–0.093/d) [35]. On the basis of these calculations, a sediment concentration of 1,270 nmol/g dry weight was expected to result in the accumulation of a lethal body burden in 10 d. Other concentrations were set as a decreasing geometric progression.

Mortality in both *Diporeia* sediment exposures was generally less than expected on the basis of the toxicokinetic calculations presented above. Failure of the toxicokinetic approach to predict toxicity in *Diporeia* may be due in part to our inability to achieve fluoranthene sediment concentrations equivalent to the highest nominal dose of 1,270 nmol/g dry weight (Table 1). Longer rolling times in the dosing protocol may have resulted in higher sediment concentrations. Although the question of whether the fluoranthene was partitioned onto the sediments or was scoured from the walls of the jar cannot be definitively resolved, measured total interstitial water concentrations were close to expected values (typically within a factor of two, Table 3), suggesting that the animals were not merely exposed to particulate fluoranthene. Further, homogeneity of the radiolabeled fluoranthene in the spiked sediments (coefficients of variation of the mean sediment concentrations were typically less than 10% on day 0, Table 1) suggests that the sediments were well mixed and the fluoranthene had sorbed onto the sediments. In addition, we observed no evidence of particulate fluoranthene when spiked sediments were examined under a dissecting microscope.

Although lower than expected, significant mortality was observed for *Diporeia* in two of the three highest sediment concentrations in the first experiment, up to 38% mortality at the highest sediment concentration (688 nmol/g dry weight) (Table 4). In the second experiment, 10-d mortality was lower than in the first experiment, but mortality after 30 d was significantly greater in the four highest sediment concentrations than in controls.

Estimated and measured pore-water concentrations in the *Diporeia* exposures range up to 1,760 nmol/L total or 580 nmol/L freely dissolved fluoranthene (Table 3). In contrast, no mortality was observed in two 10-d water-only fluoranthene exposures conducted in our lab for this species at concentrations up to the limit of fluoranthene's water solubility, 1,285 nmol/L [35]. Because fluoranthene was not toxic to *Diporeia* in 10-d water-only tests, observed toxicity at day 10 in the first sediment exposure is contrary to predictions based on the EqP approach. This contradiction may have resulted in part from the additional stress of the fluoranthene on senescent animals (discussed below), whereas water-only toxicity tests were conducted on younger animals. This result illustrates the complexities that can be encountered when attempting to validate the EqP approach with a compound that is not toxic in a short-term test at its limit of water solubility.

Factors other than fluoranthene sediment concentration probably contributed to the mortality observed in *Diporeia*, particularly after 17 and 30 d in the first experiment, because mortality in the controls and trace level exposures were higher

than have previously been observed in this bioassay [30]. *Diporeia* collected in December for the first experiment may have included of a greater number of senescent animals than the animals collected the following spring for the second experiment. In addition, the body burden associated with 50% mortality in the second experiment, 6.5 μmol/g wet weight, is closer to values previously observed in this species, 6 to 9 μmol/g wet weight for pyrene [13] and 6.1 μmol/g wet weight estimated for a mixture of PAHs [12]. Body burdens associated with 50% mortality in the first experiment were slightly lower (2.7 μmol/g wet weight for 10-d and 2.0 μmol/g wet weight for 17-d exposures), again suggesting that factors other than exposure to fluoranthene may have contributed to the overall mortality in experiment 1.

Apparent 17-d steady-state BSAFs for *Diporeia* exposed to trace levels of fluoranthene in the first experiment (range = 0.097–0.119) are in the range of BSAF values found for *Diporeia* exposed to trace levels of PAHs in a previous study (range = 0.056–0.21), but are generally lower than those found for trace levels of chlorinated hydrocarbons (range = 0.29–0.91) [14]. However, BSAFs for *Diporeia* exposed to a trace level in the second experiment and for concentrations other than the trace level in both experiments were higher, ranging from 0.345 to 0.818, values that are closer to the range that would be predicted on the basis of equilibrium partitioning [42]. This result suggests that limitations to the accumulation of PAHs may exist at trace levels, but not at higher concentrations. The underlying cause for this limitation remains unknown. Possible explanations include both physical/chemical interactions of the chemical with sediment that result in reduced bioavailability of compounds at trace levels, as well as physiological effects on the organism. For example, narcotics are known to cause hyperactivity at intermediate stages of analgesia in mammals [43], which might result in an increase in the rate of bioaccumulation from sediment. This interpretation is supported by the finding that maximum BSAFs and uptake clearance rates were observed after exposure to intermediate sediment concentrations, both in these experiments and in previous studies with *Diporeia* [13]. Dose-dependent changes in lipid content over time could be another important influence on BSAF calculations. Although this was not observed in the second experiment or in past studies with *Diporeia*, results from the first experiment and work with other species [44] indicate that the contribution of lipid content to BSAF calculations should be given more attention.

The EqP approach assumes that an organism receives equivalent exposure from a water-only exposure or from any phase in an equilibrated system, including pore water or by ingestion of sediment carbon. In the present experiments, *H. azteca* accumulated less fluoranthene than would be predicted on the basis of equilibrium partitioning. These results demonstrate the advantage of measuring the actual dose to the organism when estimating the bioavailability of sediment-associated contaminants. We believe that the ability of *H. azteca* to metabolize PAHs and its epibenthic lifestyle in our assay resulted in reduced accumulation of fluoranthene from sediment exposures. In our system, as in standard sediment toxicity tests that exchange water, overlying water is probably not in equilibrium with the pore water, and assumptions of the EqP approach are presumably violated. The results of the present study agree with the findings of the EPA ARCS Program, which found *Diporeia* to be more sensitive than *H. azteca* in 28-d sediment survival bioassays [45]. The suitability of *H. azteca* as a test organism should continue to be tested in a variety of sediments and systems. In contrast, *Diporeia* exhibited toxicity after 10-d exposures to sediment-associated fluoranthene that was in excess of that observed in 10-d water-only exposures. Thus, the EqP approach would have predicted no mortality and was underprotective in that experiment. However, in the first *Diporeia* experiment, seasonal factors such as senescence may have contributed to the observed mortality, whereas in the second experiment, significant mortality was observed only after 30 d, a time point that is not directly comparable to 10-d water-only exposures. For both organisms, the internal dose based on body residue was a more reliable indicator of toxicity than EqP predictions.

Acknowledgement—This work was funded in part through interagency agreement DW 13936659-01-0 between the U.S. Environmental Protection Agency and the National Oceanic and Atmospheric Administration, but it may not necessarily reflect the views of the agencies; and no official endorsement should be inferred. Mention of trade names or commercial products does not constitute endorsement or recommendation for use. S. Kane Driscoll was supported by a National Research Council fellowship. We wish to thank R. Swartz, B. Boese, D. Shults, J. Jones, and T. Fuji for assistance in dosing the sediment; and F. Lo, E. Tigue, P. Van Hoof and J. Hsieh for technical support; T. Johengen and W. Faust for ammonia analyses; D. Gossiaux for *Diporeia* collection; C. Ingersoll and E. Greer of the National Biological Service for the gift of the *H. azteca*; and C. Ingersoll for review of a previous draft of this manuscript. We also wish to thank the Sediment Working Group, D. Di Toro, D. Hansen, W. Berry, G. Ankley, R. Hoke, H. Lee, and M. Reiley for helpful discussion.

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